

5. The method of claim 4, wherein said at least a first enzyme is uracil DNA glycosylase and said at least a second enzyme is endonuclease V.

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4. The method of claim 3, wherein said template is created by generating a substantially double-stranded nucleic acid comprising at least a first uracil residue, and contacting said nucleic acid with a combined effective amount of a first, uracil DNA glycosylase enzyme and a second, acid endonuclease IV enzyme or endonuclease V enzyme.

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2. The method of claim 1, comprising creating a substantially double-stranded nucleic acid template comprising at least a first break on only one strand.

3. The method of claim 1, wherein said template is created by contacting a substantially double-stranded nucleic acid with a combined effective amount of at least a first and second breaking enzyme combination.

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### WHAT IS CLAIMED IS:

30 13. The method of claim 12, wherein said specific cleavage composition comprises a triple helix forming composition.

25 12. The method of claim 8, wherein said template is created by contacting a substantially double-stranded nucleic acid with an effective amount of a specific cleavage composition.

20 11. The method of claim 10, wherein said specific nucleic enzyme is  $\lambda$  endonuclease.

15 10. The method of claim 9, wherein said specific nucleic enzyme is  $\lambda$  endonuclease,  $\lambda$  endonuclease or a restriction endonuclease.

10 9. The method of claim 8, wherein said template is created by contacting a substantially double-stranded nucleic acid with an effective amount of at least a first specific nucleic enzyme.

5 8. The method of claim 1, comprising creating a substantially double stranded nucleic acid template comprising at least a first specific break on at least one strand.

6. The method of claim 1, wherein said template is created by contacting a substantially double-stranded nucleic acid with an effective amount of a chemical cleavage composition.

6. The method of claim 1, wherein said template is created by contacting a substantially double-stranded nucleic acid with an effective amount of a chemical cleavage composition.

14. The method of claim 1, comprising creating a substantially double stranded nucleic acid template comprising at least a first random break on at least one strand.

15. The method of claim 1, comprising creating a substantially double stranded nucleic acid template comprising at least a first random break on only one strand.

16. The method of claim 15, wherein said template is created by generating a substantially double-stranded nucleic acid comprising a first randomly positioned exonucleic acid nucleotide, and contacting said nucleic acid with an effective amount of an exonuclease.

17. The method of claim 16, wherein said exonuclease-resistant nucleotide is a deoxyribonucleotide phosphorothioate or a deoxyribonucleotide boranoephosphate.

18. The method of claim 16, wherein said exonuclease is exonuclease III.

19. The method of claim 15, wherein said template is created by contacting a substantially double-stranded nucleic acid with an effective amount of at least a first randomly positioned exonuclease.

20. The method of claim 19, wherein said randomly-breaking nucleic enzyme is deoxyribonuclease I.

22. The method of claim 15, wherein said template is created by contacting a substantially  
restriction endonuclease.

21. The method of claim 19, wherein said randomly-breaking nucleic acid enzyme is CviII

222. The method of claim 13, wherein said template is created by contacting a substantially double-stranded nucleic acid with a combined effective amount of at least a first and second randomly-breaking enzyme combination.

24. The method of claim 23, wherein said distinct, frequent-cutting restriction endonucleases  
are selected from the group consisting of *Tsp509I*, *MaeII*, *TaiI*, *AluI*, *CviII*, *NlaIII*, *MspI*, *HpaII*,  
*BsrUI*, *BsaI*, *DpnII*, *MboI*, *Sau3A*, *DpaI*, *ChaI*, *HinfI*, *HaeIII*, *Csp6I*, *RsaI*, *TaqI* and  
*MseI*.

26. The method of claim 25, wherein said randomly-breaking chemical cleavage composition comprises or reacts to produce a hydroxyl radical.

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27. The method of claim 26, wherein said randomly-breaking chemical cleavage composition comprises a chelating agent, a metal ion, a reducing agent and a peroxide.

28. The method of claim 27, wherein said randomly-breaking chemical cleavage composition comprises EDTA, an  $Fe^{2+}$  ion, sodium ascorbate and hydrogen peroxide.

29. The method of claim 26, wherein said randomly-breaking chemical cleavage composition comprises a compound that produces a hydroxyl radical upon contact with defined wavelengths of light.

30. The method of claim 15, wherein said template is created by contacting a substantially double-stranded nucleic acid with an effective amount of gamma irradiation.

31. The method of claim 15, wherein said template is created by subjecting a substantially double-stranded nucleic acid to an effective amount of a mechanical breaking process.

32. The method of claim 31, wherein said template is created by subjecting a substantially double-stranded nucleic acid to an effective amount of a hydrodynamic force.

33. The method of claim 31, wherein said template is created by subjecting a substantially double-stranded nucleic acid to an effective amount of sonication.

40. The method of claim 39, wherein said effective polymerase is *E. coli* DNA polymerase I, *M. tuberculosis* DNA polymerase I or *Taq* DNA polymerase

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39. The method of claim 36, wherein said effective polymerase is *E. coli* DNA polymerase I, *M. thermophilic* DNA polymerase I, *H. simplex*-I DNA polymerase I, *M. radiodurans* DNA polymerase I, *T. th. DNA polymerase*, *T. th. XL* DNA polymerase, *M. tuberculosis* DNA polymerase I, *S. pneumoniae* DNA polymerase I, *T. *Taq** DNA polymerase, *D. radiodurans* DNA polymerase I, *E. coli* DNA polymerase I, *M. thermophilic* fragment, *vent* DNA polymerase, thermostable or a wild-type or modified *T7* DNA polymerase.

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38. The method of claim 36, wherein said effective polymerase has strand displacement activity.

37. The method of claim 36, wherein said effective polymerase has 5' to 3' exonuclease activity.

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36. The method of claim 1, wherein said break is a nick comprising a 3' hydroxyl group.

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35. The method of claim 15, wherein said template is created by subjecting a substantially double-stranded nucleic acid to an effective amount of freezing and thawing.

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34. The method of claim 31, wherein said template is created by subjecting a substantially double-stranded nucleic acid to an effective amount of nebulization.

41. The method of claim 1, wherein said break is a gap comprising a 3' hydroxyl group.

42. The method of claim 41, wherein said effective polymerase is *E. coli* DNA polymerase I, *Taq* DNA polymerase, *S. pneumoniae* DNA polymerase I, *T<sub>7</sub>* DNA polymerase, *D. radiodurans* DNA polymerase I, *T<sub>th</sub>* DNA polymerase, *T<sub>th</sub>* XL DNA polymerase, *M. tuberculosis* DNA polymerase I, *M. thermophilic* DNA polymerase I, *H. pylori* simplex-1 DNA polymerase, *E. coli* DNA polymerase I Klenerman, *T4* DNA polymerase, *Yeast* DNA polymerase, *E. coli* DNA polymerase I Klenow fragment, *T4* DNA polymerase, *Ven*t DNA polymerase, *M. tuberculosis* DNA polymerase I, *T<sub>4</sub>* DNA polymerase or modified *T7* DNA polymerase.

43. The method of claim 42, wherein said effective polymerase is *E. coli* DNA polymerase I, *M. tuberculosis* DNA polymerase I, *Taq* DNA polymerase or *T4* DNA polymerase.

44. The method of claim 1, wherein said terminating composition comprises a terminating deoxy nucleotide triphosphate, the base of which corresponds to said selected base.

45. The method of claim 1, wherein said terminating composition comprises a terminating deoxy nucleotide triphosphate, the base of which corresponds to said selected base.

25 46. The method of claim 1, wherein said terminating nucleotide comprises a detectable label or an isolation tag that is incorporated into said nucleic acid product.

47. The method of claim 1, wherein said template comprises a detectable label or an isolation tag.

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53. The method of claim 51, further defined as a method for mapping a nucleic acid, the method comprising detecting said nucleic acid product under conditions effective to determine the position of said nucleic acid relative to said nucleic acid product.

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52. The method of claim 51, further defined as a method for sequencing a nucleic acid, the method comprising detecting said nucleic acid product under conditions effective to determine the nucleic acid sequence of at least a portion of said nucleic acid.

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51. The method of claim 1, further comprising detecting said nucleic acid product, biotin molecule isolation tag.

50. The method of claim 1, wherein said template or said terminating nucleotide comprise a biotin molecule isolation tag.

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49. The method of claim 1, wherein said template or said terminating nucleotide comprise a radioactive, enzymatic or fluorescent label.

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48. The method of claim 1, wherein said template and said terminating nucleotide each comprise a detectable label or an isolation tag.

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(a) creating a population of substantially double-stranded nucleic acid templates comprising at least a first random break on at least one strand;

(b) contacting said templates with an effective polymerase and at least a first degradable extension-producing composition comprising three non-degradable extending nucleotides and one degradable nucleotide, under conditions and for a time effective to produce a population of degradable extension-producing composition comprising a degradable nucleotide and one degradable nucleotide, under conditions and for a time effective to produce a polymerase and at least a first non-degradable extending and terminating polymerase and at least a first non-degradable extending deoxyribonucleotides, at least one composition comprising four non-degradable extending deoxyribonucleotides, at least one of said non-degradable extending deoxyribonucleotides comprising a detectable label or an isolation tag, under conditions and for a time effective to produce a population of terminated nucleic acid products comprising a degradable region and a nondegradable region;

(c) removing said degradable extension-producing composition from contact with said templates;

(d) contacting said population of degradable nucleic acid products with an effective polymerase and at least a first non-degradable extending deoxyribonucleotides comprising a detectable label or an isolation tag, under conditions and for a time effective to produce a population of terminated nucleic acid products comprising a degradable region and a nondegradable region;

(e) amount of a degrading composition to degrade said degradable region, thereby contacting said population of terminated nucleic acid products with an effective

34. The method of claim 53, comprising:

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base.

61. The method of claim 15, comprising contacting said template with an effective nucleic acid product comprising at least one specified base prior to termination at said selected polymerase and extending and terminating composition under conditions effective to produce a

selected base.

60. The method of claim 15, wherein at least a first specified base is incorporated at the random break of said template prior to producing the nucleic acid product terminated at the

59. The method of claim 58, wherein said nucleic acid product is separated by electrophoresis, mass spectroscopy, FPLC or HPLC prior to detection.

58. The method of claim 51, wherein said nucleic acid product is separated prior to detection.

and said nucleic acid product is purified using said isolation tag prior to detection.

57. The method of claim 51, wherein said nucleic acid product comprises an isolation tag,

and said nucleic acid product is detected by detecting said label.

56. The method of claim 51, wherein said nucleic acid product comprises a detectable label,

55. The method of claim 54, wherein said degradable nucleotide comprises a uracil base, and wherein said degrading composition comprises a combined effective amount of a uracil DNA glycosylase enzyme and an endonuclease IV or an endonuclease V enzyme.

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62. The method of claim 60, comprising contacting said template with four extending nucleotides and said terminating nucleotide under conditions effective to produce a population of nucleic acid products terminated at said selected base.

63. The method of claim 62, wherein at least one of said extending nucleotides is a degradable nucleotide.

64. The method of claim 60, further defined as a method for identifying a selected dinucleotide sequence in said nucleic acid template, the dinucleotide sequence being the complement of said selected and selected base, the method comprising: blocking said template by contacting with a blocking composition comprising the three dideoxynucleotide triphosphates that do not contain the selected base; removing said blocking composition from contact with said template; contacting said template with at least a first extending and terminating composition comprising an extending dideoxynucleotide triphosphate containing said selected dinucleotide sequence in said nucleic acid product under conditions effective to produce a population of nucleic acid products terminating at said selected base; and detecting said nucleic acid product under conditions effective to identify the selected dinucleotide sequence in said nucleic acid template.

(d) detecting said nucleic acid product under conditions effective to identify the selected dinucleotide sequence in said nucleic acid template.

65. The method of claim 15, further defined as a method for identifying a selected dinucleotide sequence of a first and second base in a nucleic acid template, said method comprising:

a) blocking said template by contacting with a blocking composition comprising three dideoxynucleotide triphosphates that do not contain the complement of said first base;

b) removing said blocking composition from contact with said template;

c) contacting said template with at least a first extending and terminating composition comprising an extending dideoxynucleotide triphosphate containing the complement of said first base, and a tagged or labeled terminating dideoxynucleotide triphosphate containing the complement of said second base, under conditions effective to produce a nucleic acid product terminating with a dideoxynucleotide triphosphate containing the complement of said second base, said nucleotide sequence complementary to said first and second base; and

d) detecting said nucleic acid product under conditions effective to identify said selected dinucleotide sequence in said nucleic acid template.

66. The method of claim 65, wherein step (c) comprises contacting said template with a single extending and terminating composition that comprises both said extending dideoxynucleotide triphosphate and said terminating dideoxynucleotide triphosphate.

67. The method of claim 65, wherein step (c) comprises first contacting said template with an extending composition that comprises said extending dideoxynucleotide triphosphate, and then contacting said template with a terminating composition that comprises said terminating dideoxynucleotide triphosphate.

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68. The method of claim 67, wherein step (c) comprises, in sequence, contacting said template with an extending composition that comprises said extending deoxyribonucleotide triphosphate, removing said extending composition from contact with said template, contacting said template with a distinct terminating composition that comprises said terminating deoxyribonucleotide triphosphate, removing said extending composition from contact with said template, and contacting said template with a distinct terminating composition that comprises said terminating deoxyribonucleotide triphosphate, removing said extending composition from contact with said template, and incorporating at said random break of said template prior to producing said nucleic acid product.

69. The method of claim 15, wherein at least a first and a second specified base are incorporated at said random break of said template prior to producing said nucleic acid product.

70. The method of claim 15, comprising subjecting said template to a series of blocking and extending reactions prior to contact with said terminating composition, thereby producing an extended nucleic acid product comprising a series of additional bases preceding the selected terminating base.

71. The method of claim 69, further defined as a method for identifying a selected triphosphate sequence in said acidic nucleic acid template, the triphosphate sequence being the complement of said first and second specified bases and said selected base, the method comprising:

20 a) blocking said template by contacting with a first blocking composition comprising three deoxyribonucleotide triphosphates that do not contain the first specified base;

25 b) removing said first blocking composition from contact with said template;

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first base;

three deoxyribonucleotide triphosphates that do not contain the complement of said blocking said template by contacting with a first blocking composition comprising

a)

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trinucleotide sequence of a first, second and third base in a nucleic acid template, said method selecting triphosphate further defined as a method for identifying a selected

selected triphosphate sequence in said nucleic acid sample.

b) detecting said nucleic acid product under conditions effective to identify a

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second specified bases and said selected base; and nucleic acid product terminating with a triphosphate sequence of said first and triphosphate containing said selected base, under conditions effective to produce a said second specified base, and a tagged or labeled terminating deoxyribonucleotide containing composition comprising an extending deoxyribonucleotide terminating contacting said template with a first extending and terminating

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removing said second blocking composition from contact with said template;

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c) blocking said template by contacting with a second blocking composition comprising three deoxyribonucleotide triphosphates that do not contain the second specified base;

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removing said first extending composition from contact with said template;

d) extending said template by contacting with a first extending composition comprising an extending deoxyribonucleotide triphosphate containing said first specified base;

c)

extending said template by contacting with a first extending composition

comprising an extending deoxyribonucleotide triphosphate containing said first

specifying an extending deoxyribonucleotide triphosphate containing said first

2) detecting said nucleic acid product under conditions effective to identify said selected dinucleotide sequence in said nucleic acid sample.

3) contacting said template with at least a first extending and terminating dinucleotide sequence complementary to said first, second and third bases; and under conditions effective to produce a nucleic acid product terminating with a diideoxynucleotide triphosphate containing the complement of said third base, the complement of said second base, and a tagged or labeled terminating composition comprising an extending deoxynucleotide triphosphate containing contacting said template with at least a first extending and terminating dinucleotide sequence complementary to said first, second and third bases; and under conditions effective to produce a nucleic acid product terminating with a diideoxynucleotide triphosphate containing the complement of said third base, the complement of said second base, and a tagged or labeled terminating composition comprising an extending deoxynucleotide triphosphate containing

4) removing said blocking composition from contact with said template; comprising three diideoxynucleotide triphosphates that do not contain the complement of said second base;

5) extending said template by contacting with a second blocking composition comprising an extending deoxynucleotide triphosphate containing the complement of said first base;

6) removing said first extending composition from contact with said template;

7) blocking said template by contacting with a second blocking composition comprising three diideoxynucleotide triphosphates that do not contain the complement of said second base;

8) removing said second blocking composition from contact with said template;

9) detecting said dinucleotide sequence in said nucleic acid sample.

b) removing said first blocking composition from contact with said template;

c) extending said template by contacting with a first extending composition comprising an extending deoxynucleotide triphosphate containing the complement of said first base;

d) removing said first extending composition from contact with said template;

e) blocking said template by contacting with a second blocking composition comprising three diideoxynucleotide triphosphates that do not contain the complement of said second base;

## 73. The method of claim 72, comprising:

a) blocking said template by contacting with a first blocking composition comprising three dideoxyribonucleotide triphosphates that do not contain the complement of said first base;

b) removing said first blocking composition from contact with said template;

c) extending said template by contacting with a first extending composition comprising an extending deoxyribonucleotide triphosphate containing the complement of said first base;

d) removing said first extending composition from contact with said template;

e) blocking said template by contacting with a second blocking composition comprising three dideoxyribonucleotides triphosphates that do not contain the complement of said second base;

f) removing said second blocking composition from contact with said template;

g) further extending said template by contacting with a second extending composition comprising an extending deoxyribonucleotide triphosphate containing the complement of said second base;

h) terminating the reaction by contacting said template with a terminating composition comprising a triphosphate containing the complement of said third base; and

77. The method of claim 1, wherein each of said four terminating bases comprise a distinct fluorescent label.

78. The method of claim 1, wherein said template is a covalently closed circular template.

75. The method of claim 74, further defined as a method for sequencing a nucleic acid comprising detecting said population of nucleic acid products terminated at four selected bases under conditions effective to determine the sequence of at least a portion of said nucleic acid.

76. The method of claim 75, further comprising contacting said template with at least four extending nucleotides.

detecting said nucleic acid product under conditions effective to identify a selected dinucleotide sequence in said nucleic acid sample.

method.

85. The method of claim 81, wherein said template is created by an isothermal amplification

b) conducting a polymerase chain reaction to create said template.

base; and

wherein at least one of said first or second primers comprises at least a first uracil amplitify said template when used in conjunction with a polymerase chain reaction, contacting said precursor molecule with at least a first and a second primer that

84. The method of claim 83, comprising:

83. The method of claim 82, wherein said template is created by PCR.

amplification method.

82. The method of claim 81, wherein said template is created by a temperature cycling

a precursor nucleic acid molecule.

81. The method of claim 1, wherein said template is created by amplifying the template from

nucleic acid molecule.

80. The method of claim 1, wherein said template is created by cleavage from a precursor

86. A method for sequencing a nucleic acid molecule, comprising:

a) creating a population of substantially double-stranded nucleic acid molecules from said nucleic acid molecule, each of said templates comprising at least a first random break on at least one strand;

b) contacting said templates with an effective polymerase and a terminating under conditions effective to produce a population of terminated nucleic acid composition comprising four distinct labeled or tagged terminating nucleotides, determining said terminated nucleic acid products under conditions effective to detecting said terminated nucleic acid products under conditions effective to determine the nucleic acid sequence of at least a portion of said nucleic acid molecule.

c) detecting said terminated nucleic acid products under conditions effective to determine the nucleic acid sequence of at least a portion of said nucleic acid composition in four distinct reactions, each of said reactions comprising only one of said four distinct labeled or tagged terminating nucleotide.

88. The method of claim 86, wherein said templates are contacted with said terminating composition in a single reaction, and wherein each of said four terminating nucleotides comprises a distinct, fluorescent label.

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89.

A method for sequencing a nucleic acid molecule, comprising:

a) creating at least a first substantially double-stranded nucleic acid template from said nucleic acid molecule, the template comprising at least a first random break on at least one strand;

b) contacting said template with an effective polymerase and at least a first extending

c) determining said terminanted nucleic acid products under conditions effective to determine the nucleic acid sequence of at least a portion of said nucleic acid molecule.

90. A method of sequencing a nucleic acid molecule by identifying at least a selected dinucleotide sequence comprising at least a first base and a second base, the method comprising:

a) creating a population of substantially double-stranded nucleic acid template from said nucleic acid molecule, the templates each comprising a selected dinucleotide sequence on a template strand and comprising at least a first random break on a non-template strand;

b) blocking said templates by contacting with a blocking composition comprising three deoxyribonucleotide triphosphates that do not contain the complement of said first base;

c) removing said blocking composition from contact with said templates;

complement of said second base;

comprising three deoxyribonucleotide triphosphates that do not contain the blocking said template by contacting with a second blocking composition

iii)

removing said first extending composition from contact with said template;

complement of said first base;

extending said template by contacting with a first extending composition comprising an extending deoxyribonucleotide triphosphate containing the

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first, second and third base, wherein step (d) of said method comprises: molecule comprising identifying at least a selected dinucleotide sequence comprising at least a nucleic acid

91. The method of claim 90, further defined as a method for sequencing a nucleic acid

comprising the identified dinucleotide sequences to determine the contiguous nucleic acid sequence of at least a portion of said nucleic acid molecule.

i)

detecting said nucleic acid products under conditions effective to identify said selected dinucleotide sequence in said nucleic acid template; and

complementarity to said first and second base;

which the non-template strands terminate with a dinucleotide sequence under conditions effective to produce a population of nucleic acid products in

deoxyribonucleotide triphosphate containing the complement of said second base,

the complement of said first base and a tagged or labeled terminating composition comprising an extending deoxyribonucleotide triphosphate containing

containing said template with at least a first extending and terminating

d)

30) containing said population of degradable nucleic acid products with an effective polymerase and at least a first nondegradable extending and terminating contacting said population of degradable nucleic acid products with an effective

25) removing said degradable extension-producing composition from contact with said templates;

20) comprising said degradable nucleotide; contacting said degradable nucleotide with an effective polymerase and at least a first degradable extension-producing composition comprising three non-degradable degrading deoxyribonucleotides and one degradable nucleotide, under conditions and extending deoxyribonucleotides and one degradable nucleotide, under conditions and removing said degradable extension-producing composition from contact with said templates;

15) creating a population of substantially double-stranded nucleic acid templates from said nucleic acid comprising at least a first, random break on only one strand;

10) compiling to generate at least a contiguous portion of the sequence of said nucleic acid molecule. and wherein the selected trinucleotide sequences of the template strand are identified and

5) contacting said templates with at least a first extending and terminating composition comprising an extending deoxyribonucleotide triphosphate containing the complement of said second base, and a tagged or labeled terminating deoxyribonucleotide triphosphate containing the complement of said third base, which the non-template strands terminate with a trinucleotide sequence under conditions effective to produce a population of nucleic acid products in complementary to said first, second and third bases;

92. A method of mapping a nucleic acid, comprising:

iv) removing said first blocking composition from contact with said templates;

v) contacting said templates with at least a first extending and terminating

composition comprising an extending deoxyribonucleotide triphosphate containing the complement of said second base, and a tagged or labeled terminating deoxyribonucleotide triphosphate containing the complement of said third base, which the non-template strands terminate with a trinucleotide sequence under conditions effective to produce a population of nucleic acid products in complementary to said first, second and third bases;

10) creating a population of substantially double-stranded nucleic acid templates from said nucleic acid comprising at least a first, random break on only one strand;

15) removing said degradable extension-producing composition from contact with said templates;

20) comprising said degradable nucleotide; contacting said degradable nucleotide with an effective polymerase and at least a first degradable extension-producing composition comprising three non-degradable degrading deoxyribonucleotides and one degradable nucleotide, under conditions and extending deoxyribonucleotides and one degradable nucleotide, under conditions and removing said degradable extension-producing composition from contact with said templates;

25) creating a population of substantially double-stranded nucleic acid templates from said nucleic acid comprising at least a first, random break on only one strand;

30) removing said degradable extension-producing composition from contact with said templates;

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5. composition comprising four non-degradable extending deoxyribonucleotides, at least one of said non-degradable extending deoxyribonucleotides comprising a detectable label or an isolation tag, under conditions and for a time effective to produce a population of terminated nucleic acid products comprising a degradable region and a nondegradable region;

10. (e) contacting said population of terminated nucleic acid products with an effective amount of a degrading composition to degrade said degradable region, thereby producing nested nucleic acid products; and

15. (f) detecting said nested nucleic acid products under conditions effective to determine the position of said nucleic acid relative to said nucleic acid product.

20. (a) creating a substantially double-stranded nucleic acid template comprising at least a first random break on at least one strand, a selected dinucleotide sequence on a template strand and comprising an exonuclease-resistant nucleotide in the non-template strand, wherein the base of said exonuclease-resistant nucleotide is complementary to said first base;

25. (b) contacting said template with an amount of an exonuclease effective to degrade the non-template strand until the position of the exonuclease-resistant nucleotide;

30. (c) removing said exonuclease from contact with said template;

(d) contacting said template with at least a first terminating composition comprising a tagged or labeled terminating deoxyribonucleotide triphosphate containing the contacting said template with at least a first terminating composition comprising a tagged or labeled terminating deoxyribonucleotide triphosphate containing the

30 95. A method of determining the length of a single-stranded overhang of a telomere, comprising contacting a telomere comprising a single-stranded overhang under conditions effective to allow primer that hybridizes to said single-stranded overhang under conditions effective to allow at least a portion of said subtelomeric region.

25 c) detecting said nucleic acid product under conditions effective to determine the nucleic acid sequence of said telomeric overhang, said telomeric repeat region and detecting said nucleic acid product under conditions effective to determine the at least a portion of said subtelomeric region.

20 b) contacting said nucleic acid with a composition comprising a primer that hybridizes to said single-stranded telomeric overhang, an effective polymerase, four extending nucleotides and at least a first tagged or labeled terminating nucleotide under conditions effective to produce a nucleic acid product extending from said primer into said subtelomeric region; and

15 a) providing a substantially double-stranded nucleic acid that comprises, in order, a terminal single-stranded telomeric overhang, a double-stranded telomeric repeat region and a double-stranded subtelomeric region;

10 94. A method of sequencing through a telomeric repeat region into a subtelomeric region, comprising:

5 e) detecting said nucleic acid product under conditions effective to identify said selected dinucleotide sequence in the template strand of said nucleic acid template.

and second base; and

acid product terminating with a dinucleotide sequence complementary to said first complement of said second base, under conditions effective to produce a nucleic acid product terminating with a dinucleotide sequence complementary to said first template.

5. The method of claim 95, further comprising contacting the primers hybridized to said single-stranded overhang with a ligation composition in an amount and for a time effective to ligate said primers, and wherein the length of the ligated primers is quantitated.

96. The method of claim 95, further comprising contacting the primers hybridized to said single-stranded overhang with a ligation composition in an amount and for a time effective to create a substantially double stranded nucleic acid template comprising at least a first break on creating a substantially double stranded nucleic acid template comprising at least a first strand, and contacting said template with at least one strand, and contacting said template with:

97. A method of selecting a nucleic acid product terminated at a selected base, comprising creating a substantially double stranded nucleic acid template comprising at least a first break on terminating a nucleotide, wherein the base of said terminating nucleotide corresponds to said selected base.

10. 97. A method of selecting a nucleic acid product terminated at a selected base, comprising an effective polymerase and an extending composition comprising a template that terminates at said selected base.

15. a) an effective polymerase and a terminating composition comprising a template comprising at least a first break on terminating a nucleotide, wherein the base of said terminating nucleotide corresponds to said selected base, and an effective polymerase and an extending composition under conditions effective to produce a fully extended product only from a template that terminates at said selected base; or

b) an effective polymerase and an extending composition under conditions effective to produce a template comprising at least a first random double stranded break.

20. 98. The method of claim 97, comprising creating a substantially double stranded nucleic acid template comprising at least a first random double stranded break.

25. 98. The method of claim 97, comprising creating a substantially double stranded nucleic acid template comprising at least a first random double stranded break.



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100. The method of claim 98, further defined as a method for determining the position of a selected dinucleotide sequence of a first, second and third base in a nucleic acid template, said ligating a double-stranded nucleic acid segment to said double-stranded break, detecting said nucleic acid product under conditions effective to determine the position of said selected dinucleotide sequence in said nucleic acid sample.

10 i) detecting said nucleic acid product under conditions effective to determine the position of said selected dinucleotide sequence in said nucleic acid sample.

15 a) ligating a double-stranded nucleic acid segment to said double-stranded break, said double-stranded nucleic acid segment comprising an upper strand a 5', end comprising a phosphate group and a blocked 3', end and a lower strand comprising a blocked 5', end and a 3', end comprising a hydroxyl group;

b) blocking said template by contacting with a first blocking composition comprising three diideoxynucleotide triphosphates that do not contain the complement of said first base;

20 c) removing said first blocking composition from contact with said template;

d) extending said template by contacting with a first extending composition comprising a triphosphate containing deoxyribonucleotide triphosphate containing the complement of said first base;

25 e) removing said first extending composition from contact with said template;

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m) detecting said nucleic acid product under conditions effective to determine the position of said selected dinucleotide sequence in said nucleic acid sample.

l) contacting said template with at least a third extending composition comprising four extending deoxyribonucleotide triphosphates, at least one of said extending deoxyribonucleotide triphosphates containing a tagged or labeled base, under conditions effective to produce a fully extended tagged or labeled nucleic acid product with a dinucleotide sequence complementary to said first, second and third bases; and

j) blocking said template by contacting with a third blocking composition comprising three dideoxyribonucleotide triphosphates that do not contain the complement of said third base;

i) removing said second extending composition from contact with said template;

h) extending said template by contacting with a second extending composition comprising an extending deoxyribonucleotide triphosphate containing the complement of said second base;

g) removing said second blocking composition from contact with said template;

f) blocking said template by contacting with a second blocking composition comprising three dideoxyribonucleotide triphosphates that do not contain the complement of said second base;

101. The method of claim 98, further defined as a method of determining the position of a selected dinucleotide sequence comprising a first base and a second base in a nucleic acid sequence, the method comprising: attaching a double-stranded nucleic acid segment to said double-stranded break, said double-stranded nucleic acid segment comprising an upper strand comprising a 5' end comprising a phosphate group and a blocked 3' end; heating said template at a temperature effective to disassociate said lower strand comprising a blocked 5' end and a blocked 3' end; annealing a single-stranded oligonucleotide comprising a 3' hydroxyl group to said template, said first oligonucleotide comprising the same nucleotide sequence as said lower strand plus a first additional 3' base complementary to said first base and a second additional 3' base complementary to said second base; contacting said template with an extending composition comprising four deoxyribonucleotide triphosphates containing a tagged or labeled base, under conditions effective to produce a fully extended tagged or labeled nucleic acid product with a dinucleotide sequence complementary to said first and second bases; and detecting said nucleic acid product under conditions effective to determine the position of said selected dinucleotide sequence in said nucleic acid sample.

5) a) attaching a double-stranded nucleic acid segment to said double-stranded break, said double-stranded nucleic acid segment comprising an upper strand comprising a 5' end comprising a phosphate group and a blocked 3' end; heating said template at a temperature effective to disassociate said lower strand comprising a blocked 5' end and a blocked 3' end; annealing a single-stranded oligonucleotide comprising a 3' hydroxyl group to said template, said first oligonucleotide comprising the same nucleotide sequence as said lower strand plus a first additional 3' base complementary to said first base and a second additional 3' base complementary to said second base;

10) b) heating said template at a temperature effective to disassociate said lower strand of said adaptor;

15) c) annealing a single-stranded oligonucleotide comprising a 3' hydroxyl group to said template, said first oligonucleotide comprising the same nucleotide sequence as said lower strand plus a first additional 3' base complementary to said first base and a second additional 3' base complementary to said second base;

20) d) contacting said template with an extending composition comprising four deoxyribonucleotide triphosphates containing a tagged or labeled base, under conditions effective to produce a fully extended tagged or labeled nucleic acid product with a dinucleotide sequence complementary to said first and second bases;

25) e) detecting said nucleic acid product under conditions effective to determine the position of said selected dinucleotide sequence in said nucleic acid sample.

102. The method of claim 98, further defined as a method of determining the position of a selected tri-nucleotide sequence comprising a first base, a second base and a third base in a nucleic acid template, the method comprising:

e) detecting said nucleic acid product under conditions effective to determine the position of said selected tri-nucleotide sequence in said nucleic acid sample.

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20 d) contacting said template with an extending composition comprising four deoxyribonucleotide triphosphates containing a tagged or labeled base, under conditions effective to produce a fully extended tagged or labeled nucleic acid product with a tri-nucleotide sequence complementary to said first, second and third bases; and

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15 c) annealing a single-stranded oligonucleotide comprising a 3' hydroxyl group to said template, said first oligonucleotide comprising the same nucleotide sequence as said lower strand plus a first additional 3' base complementary to said first base, a second additional 3' base complementary to said second base and a third additional 3' base complementary to said third base;

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10 b) heating said template at a temperature effective to disassociate said lower strand of said adapter;

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5 a) attaching a double-stranded nucleic acid segment to said double-stranded break, said double-stranded nucleic acid segment comprising an upper strand comprising a 5' end comprising a phosphate group and a blocked 3' end; and

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nuclieic acid template, the method comprising:

selecting tri-nucleotide sequence comprising a first base, a second base and a third base in a nucleic acid template, the method comprising:

102. The method of claim 98, further defined as a method of determining the position of a selected tri-nucleotide sequence comprising a first base, a second base and a third base in a nucleic acid template, the method comprising:

103. The method of claim 98, further defined as a method of determining the position of a selected dinucleotide sequence comprising a first base and a second base in a nucleic acid template, the method comprising:

a) ligating a double-stranded nucleic acid segment to said double-stranded break, said double-stranded nucleic acid segment comprising a phosphate group and a 5', end and a 3', end;

b) heating the ligated double-stranded nucleic acid segment at a temperature effective to disassociate said lower strand of said adapter;

c) annealing a first single-stranded oligonucleotide comprising a 3', hydroxyl group to said templates, said first oligonucleotide comprising the same nucleotide sequence as said lower strand;

d) blocking said templates by contacting with a first blocking composition comprising a dideoxyribonucleotide triphosphate that contains the complement of said blocking sequence by contacting with a first blocking composition comprising a deoxyribonucleotide triphosphate, one of said deoxyribonucleotide triphosphates comprising a uracil base, under conditions effective to completely extend the non-contacting said templates with at least a first extending composition comprising

e) removing said first blocking composition from contact with said templates;

f) contacting said templates with a second extending composition comprising a deoxyribonucleotide triphosphate, one of said deoxyribonucleotide triphosphates comprising a uracil base, under conditions effective to completely extend the non-contacting said templates with at least a second extending composition comprising a deoxyribonucleotide triphosphate, one of said deoxyribonucleotide triphosphates comprising a uracil base;

g) heating the templates at a temperature effective to disassociate said first single strand; and

25 h) heating the templates at a temperature effective to disassociate said first single stranded oligonucleotide.

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annexaline a second single-stranded oligonucleotide comprising a 3' hydroxyl group blocking said templates by contacting with a second blocking composition comprising a di-deoxyribonucleotide triphosphate that contains the complement of said second base;

removing said second blocking composition from contact with said templates;

contacting said templates with said at least a first extending composition comprising four deoxyribonucleotide triphosphates, one of said deoxyribonucleotide triphosphates comprising a uracil base, under conditions effective to completely extend the non-template strand;

heating the templates at a temperature effective to dissociate said single stranded oligonucleotide;

annealing a third single-stranded oligonucleotide comprising a 3' hydroxyl group to said templates, said second oligonucleotide comprising the same nucleotide sequence as said second single-stranded oligonucleotide plus a second additional nucleotide to said templates, said second oligonucleotide comprising the same nucleotide sequence as said second single-stranded oligonucleotide to said templates, at least a second extending composition comprising four deoxyribonucleotide triphosphates, at least one of which comprises a detectable label, under conditions effective to completely extend the non-template strand;

3' base complementary to said second base;

removing said first blocking composition from contact with said templates;

first base;

comprising a dideoxynucleotide triphosphate that contains the complement of said blocking said templates by contacting with a first blocking composition sequence as said lower strand;

to said templates, said first oligonucleotide comprising the same nucleotide annealing a first single-stranded oligonucleotide comprising a 3'-hydroxyl group effective to disassociate said lower strand of said adaptor;

heating the ligated double-stranded nucleic acid segment at a temperature comprising a blocked 5' end and a blocked 3' end;

said double-stranded nucleic acid segment comprising an upper strand comprising a 5' end comprising a phosphate group and a blocked 3' end and a lower strand nucleic acid template, the method comprising:

selected dinucleotide sequence comprising a first base, a second base and a third base in a ligating a double-stranded nucleic acid segment to said double-stranded break, heating the ligated double-stranded nucleic acid segment to said double-stranded break, annealing a first single-stranded oligonucleotide comprising a 3'-hydroxyl group effective to disassociate said lower strand of said adaptor;

detecting said nucleic acid products under conditions effective to determine the position of said selected dinucleotide sequence in said nucleic acid templates.

conditions effective to degrade the non-template strands containing a uracil base, contacting said template with at least a first degrading composition under and determining said nucleic acid products under conditions effective to determine the position of said selected dinucleotide sequence in said nucleic acid templates.

104. The method of claim 98, further defined as a method of determining the position of a nucleic acid template, the method comprising:

selecting dinucleotide sequence comprising a first base, a second base and a third base in a nucleic acid template, the method comprising:

detecting said nucleic acid products under conditions effective to determine the position of said selected dinucleotide sequence in said nucleic acid templates;

removing said first blocking composition from contact with said templates;

first base;

comprising a dideoxynucleotide triphosphate that contains the complement of said blocking composition by contacting with a first blocking composition sequence as said lower strand;

to said templates, said first oligonucleotide comprising the same nucleotide annealing a first single-stranded oligonucleotide comprising a 3'-hydroxyl group effective to disassociate said lower strand of said adaptor;

heating the ligated double-stranded nucleic acid segment at a temperature comprising a blocked 5' end and a blocked 3' end;

said double-stranded nucleic acid segment comprising an upper strand comprising a 5' end comprising a phosphate group and a blocked 3' end and a lower strand nucleic acid template, the method comprising:

selected dinucleotide sequence comprising a first base, a second base and a third base in a ligating a double-stranded nucleic acid segment to said double-stranded break, heating the ligated double-stranded nucleic acid segment to said double-stranded break, annealing a first single-stranded oligonucleotide comprising a 3'-hydroxyl group effective to disassociate said lower strand of said adaptor;

detecting said nucleic acid products under conditions effective to determine the position of said selected dinucleotide sequence in said nucleic acid templates.

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to said templates, said second oligonucleotide comprising the same nucleotide  
m) annealing a third single-stranded oligonucleotide comprising a 3', hydroxyl group

stranded oligonucleotide;

heating the templates at a temperature effective to disassociate said second single

strand the non-template strand;

triphasphates comprising a uracil base, under conditions effective to completely  
comprising four deoxyribonucleotide tripphasphates, one of said deoxyribonucleotide  
composition

removing said second blocking composition from contact with said templates;

second base;

blocking said templates by contacting with a second blocking composition  
comprising a deoxyribonucleotide tripphosphate that contains the complement of said

additional 3', base complementary to said first base;  
h) annealing a second single-stranded oligonucleotide comprising a 3', hydroxyl  
group to said templates, said second oligonucleotide comprising the same  
nucleotide sequence as said first single-stranded oligonucleotide plus a first

stranded oligonucleotide;

heating the templates at a temperature effective to disassociate said first single

template strand;

comprising a uracil base, under conditions effective to completely extend the non-  
four deoxyribonucleotide tripphasphates, one of said deoxyribonucleotide tripphasphates  
composition

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position of said selected dinucleotide sequence in said nucleic acid templates.

(s) detecting said nucleic acid products under conditions effective to determine the

conditions effective to degrade the non-template strands containing a uracil base;

(t) contacting said templates with at least a first degrading composition under

detectable label, under conditions effective to completely extend the non-template  
comprising four deoxyribose triphosphates, at least one of which comprises a  
contacting said templates with at least a third extending and labeling composition  
detectable label, under conditions effective to completely extend the non-template

sequence as said third single-stranded oligonucleotide plus a third additional 3',  
to said templates, said second oligonucleotide comprising the same nucleotide  
annelling a fourth single-stranded oligonucleotide comprising a 3', hydroxyl group  
base complementary to said third base;

(p) annealing a fourth single-stranded oligonucleotide comprising a 3', hydroxyl group  
base complementary to said third base;

(o) heating the templates at a temperature effective to disassociate said single  
stranded oligonucleotide;

extending the non-template strands;  
comprising four deoxyribose triphosphates, one of said deoxyribose triphosphates  
comprising a uracil base, under conditions effective to completely  
contacting said templates with said at least a second extending composition

3', base complementary to said second base;  
sequence as said second single-stranded oligonucleotide plus a second additional